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Effects of Roux-en-Y gastric bypass in male Wistar rats on fat preference, eating and bone metabolism

Inaugural-Dissertation

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Summary

Today, obesity is a major health problem and is associated with a large number of co-morbidities, like cardiovascular diseases, hypertension and type 2 diabetes mellitus. Thus, severe obesity does not only impair patients' quality of life, but also places a large financial burden on our health care systems. Several treatment possibilities are available, from strict diet plans and increased exercise, to treatment with drugs. However, the only long-term effective treatment available so far is bariatric surgery, of which Roux-en-Y gastric bypass is referred to as the gold standard. Roux-en-Y gastric bypass (RYGB) is most successful in achieving long-term weight reduction, mainly by reducing eating and increasing energy expenditure. Furthermore, gastric bypass markedly alters the eating behavior of patients, i.e. the food preference is changed, shifting away from high fat and high sugar diets to less calorically dense foods.

The main aim of this study was to investigate whether these changes in preference, especially fat preference, are due to altered taste sensation. Therefore, rat models of gastric bypass were tested for spontaneous preference for different fat emulsions, with only minimal influence of post-absorptive effects by using a so-called Davis Rig setup. Furthermore, the role of conditioned taste aversion in reduced fat ingestion was tested. In a third experiment post-absorptive effects were included by measuring ad libitum fat intake over 48 hours. Our data demonstrated that altered fat preferences are probably not due to changes in taste, but likely to post-absorptive effects.

To further understand the mechanisms underlying the changes in eating behavior, the hormone status of our rats was checked. We confirmed with our rat model that the levels of the adiposity hormone leptin were decreased while the concentration of PYY, which induces satiation, was elevated.

Finally, we investigated the changes in bone density after gastric bypass. Former studies indicated that bone density may be reduced after bypass surgery beyond levels than can be explained just by the reduced body weight after surgery. To test this hypothesis, bone density measurements of bypass and control rats were done, including some control rats that were matched in body weight to bypass rats. In fact, we showed that bypass rats had markedly

lower bone densities, and that this was not seen in body weight matched rats which had lost weight just by food restriction.

Zusammenfassung

Starkes Übergewicht ist heutzutage eines der wichtigsten Gesundheitsprobleme und ist mit verschiedenen Begleiterkrankungen, wie zum Beispiel Krankheiten des Herz-Kreislaufsystems, Bluthochdruck und Typ 2 Diabetes mellitus assoziiert. Somit beeinträchtigt starkes Übergewicht nicht nur die Lebensqualität der jeweiligen Patienten, sondern stellt auch eine starke finanzielle Belastung unseres Gesundheitssystems dar. Bis heute gibt es verschiedene mögliche Behandlungsstrategien, angefangen bei strengen Diätplänen, über den Einsatz verschiedener Pharmaka bis hin zu sogenannten bariatrischen Operationen, wobei von diesen der Roux-en-Y Gastric Bypass (Gastric Bypass) als Goldstandart angesehen wird. Der Gastric Bypass ist die zurzeit erfolgreichste Behandlungsmethode, um eine anhaltende Gewichtsreduktion zu erzielen. Die Wirkmechanismen dieser Operation sind derzeit noch nicht vollständig verstanden, aber die Beobachtung, dass die Operation das Essverhalten der Patienten nachhaltig ändert, kann als gesichert angesehen werden. So wird die Präferenz für Nahrungsmittel mit hohem Fett- und Zuckergehalt zu einer Bevorzugung von Lebensmitteln mit niedrigerem Kaloriengehalt verschoben.

Das Hauptziel dieser Studie ist die Fragestellung, ob die veränderte Nahrungsselektion nach Gastric Bypass Operation, auf einer Veränderung der Geschmackswahrnehmung basiert. Hierfür wurden Ratten als Modellsystem genutzt, deren Fettkonsumverhalten unter Ausschluss postabsorptiver Effekte, in einem Davis Rig getestet wurden. Des Weiteren wurde die spontane Fettpräferenz mit einer durch klassische Konditionierung erzielten Fettaversion verglichen. Außerdem wurde in einem dritten Experiment die freie Fettaufnahme über 48 Stunden gemessen.
Insgesamt konnte gezeigt werden, dass die Veränderung der Fettselektion nicht auf Geschmackskomponenten, sondern auf Veränderung der postabsorptiven Effekte zu beruhen scheint.

Zum tieferen Verständnis der verschiedenen Komponenten, die schlussendlich zu den Veränderungen im Essverhalten führen, wurden die postprandialen Spiegel verschiedener gastrointestinaler Hormone gemessen. Es zeigte sich, dass mit sinkendem Körpergewicht, respektive Nahrungsaufnahme, der Plasmaleptinspiegel abnimmt, während die Spiegel des Sättigungshormons PYY ansteigen.

Der letzte Teil der im Rahmen dieser Dissertation durchgeführten Untersuchungen befasste sich mit den Auswirkungen der Gastric Bypass Operation auf den Knochenmetabolismus. Frühere Studien deuteten an, dass eine reduzierte Knochendichte nach Gastric Bypass nicht ausschließlich auf das reduzierte Körpergewicht der Tiere zurückgeführt werden kann. Um diese These zu überprüfen, wurden Knochendichtemessungen von Bypass Tieren und Kontrolltieren verglichen, wobei ein Teil der Kontrolltiere durch restriktive Diät auf das Körpergewicht der Bypass Tiere eingestellt wurden. Es zeigte sich, dass Gastric Bypass Ratten geringere Knochendichten aufwiesen als die Kontrolltiere, deren Körpergewicht durch Futterentzug dem der Bypass Tiere entsprach.

1 Introduction

The prevalence of obesity has risen worldwide over the last decades and is still growing [1][2] [3]. Because several co-morbidities of obesity, such as type 2 diabetes or cardiovascular diseases, impair the life quality of patients, severe obesity must be considered a serious health problem that is also present in children at alarming rates [4][5]. During the last decades many scientific projects aimed to identify the underlying causes of obesity. However, as for now, no comprehensive explanation was found and the problem seems to be caused by many factors. Complex interactions of different endocrine and central processes control the overall metabolic state of an organism. Apart from the learned and cultural factors [6] [7], which play an important role together with the nutrient composition of food and the time of eating, the endocrine system is a key mediator relaying information concerning the metabolic state of the periphery to central controllers in the brain that trigger the appropriate behavioral reactions [8].

The maintenance, gain or loss of body weight is basically a question of balance between energy uptake and expenditure. Body weight, food intake and energy expenditure interact with each other through a variety of different mechanisms. Becoming obese indicates an imbalance in energy metabolism, with energy uptake being higher than the expenditure and with the majority of extra calories being stored as fat tissue.

These imbalances may also affect the interaction between the periphery and central compartments. On one hand, direct afferent fibers of the vagus nerve and visceral afferents passing via the spinal cord pass information from the periphery to a number of centers in the brain that are involved in the control of eating and energy expenditure [9]. On the other hand, various circulating factors may reflect the peripheral status and signal to the brain either by crossing the blood brain barrier or by directly binding to receptors accessible in areas of the brain that are not protected by the blood brain barrier. The major centers involved in the control of energy balance are concentrated in the fore- and hindbrain [10].

1.1 The Physiological Situation

1.1.1 Long term acting hormones – adiposity signals

Two well-investigated adiposity signals are insulin and leptin. Leptin is mainly secreted from white adipocytes and transported via the blood stream to leptin receptors in the brain, where it alters the activity of specific hindbrain and hypothalamic neurons [11] [12]. Leptin levels increase proportionally to the amount of stored body fat [13]. Insulin is released by pancreatic beta cells; the basal and nutrient-stimuli-dependent insulin secretion rises proportionally to body fat because of the ensuing insulin resistance [14]. Both hormones have therefore higher circulating plasma levels the more fat is stored.

Leptin and insulin seem to act mainly via hypothalamic signaling by binding to receptors in the arcuate nucleus (ARC), but possibly also other areas [15] [16]. Here, catabolic pathways are stimulated, comprising pro-opiomelanocortin (POMC)- and cocaine and amphetamine regulated transcript (CART)-expressing neurons [17] [18]. Anabolic pathways are inhibited by inhibiting neuropeptide Y (NPY)- and agouti-related peptide (AGRP)-expressing neurons [19]. These neurons form connections to the paraventricular nucleus (PVN) and to the lateral hypothalamic area (LHA) [20] [21]. The information also reaches the nucleus tractus solitarius (NTS), which further integrates information and signaling from the periphery, but also from the cortex to appropriately modify behavior [22].

1.1.2 Short-term acting hormones – Satiation Signals

The feeling of satiation is mainly affected by several short-term acting hormones, which are referred to as satiation signals. These signals form a complex network of multiple interactions. Some of these hormones are also part of the incretins and therefore constitute a direct link between the control of eating and of glucose metabolism; the incretin effect refers to the elevated insulin secretion that can be observed after an oral glucose load in comparison to an isoglycemic glucose infusion [23]. Two hormones are mainly responsible to evoke the incretin effect. The glucose-dependent insulinotropic polypeptide (formerly gastrin inhibitory polypeptide, GIP) is secreted in response to intraluminal nutrients from the K cells of the small intestine [24]. Glucagon-like peptide 1 (GLP-1) is produced by the L cells of the small intestine and the colon and is also secreted due to intraluminal nutrients [25]. Beside their insulinotropic action, GIP and GLP-1 decrease food intake, inhibit proximal gastrointestinal mobility and glucagon secretion [25].

A third important satiation signal, peptide YY (PYY), is co-secreted together with GLP-1 by the L cells. PYY reduces food intake and enhances the ileal break function of GIP and GLP-1, which means that it delays proximal gastric motility [26]. PYY is a member of a protein superfamily, the PP-fold peptides. Another PP-fold member that is also involved in gastro intestinal controls is the pancreatic polypetide (PP) which is released by pancreatic islet cells and acts mainly on the exocrine part of the pancreas, the biliary function, gastric acid secretion and gut motility [26].

A fifth hormone is mainly derived from the pancreas. Amylin is a peptide hormone that is stored together with insulin in the beta cells of the pancreas, and it is released after ingestion, when blood glucose levels are changed. Its main action seems to be reduction of food intake and body weight [27]. The pancreas also releases glucagon which is secreted by the pancreatic alpha cells in response to lower blood glucose levels; glucagon restores normal glucose levels via triggering gluconeogenesis and glycogenolysis. Hence, glucagon is an important counterpart to insulin. It has been shown in knockout mouse models that a dysfunction of the glucagon signaling causes increased lipolysis and energy expenditure [28].

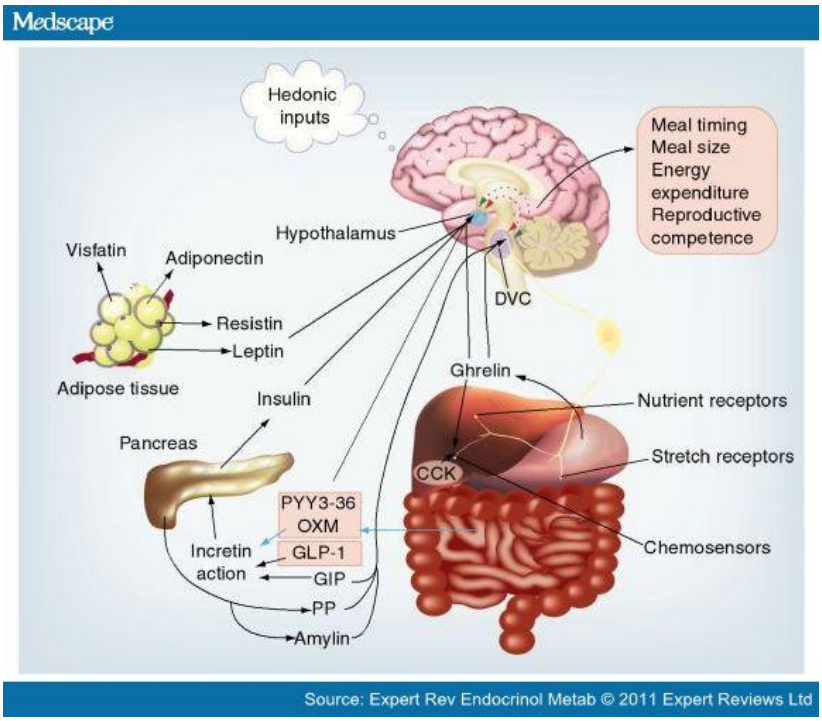

Figure 1-1: The complex hormonal network that controls energy homeostasis

The different hormones are shown that are secreted by the pancreas, fat tissue and the gastrointestinal tract, and that are integrated in the hypothalamus and the dorsal vagus complex (DVC) together with hedonic inputs to form meal associated behavioral output.

1.1.3 The sensation of taste

Taste is one of our oldest senses and enables us to distinguish our food in respect to its nutritional composition. Up to now, we know five different taste aspects, sweet, sour, salt, bitter and umami, the latter referring to the taste of glutamate [29]. Beside these individual taste aspects, there are other neurons that sense food qualities like viscosity, temperature or texture [30]. All this information passes via the nucleus of the solitary tract, the thalamus and the primary taste cortex to the orbitofrontal cortex. There, the inputs from taste receptors are integrated with the information coming from other senses like visual or olfactory inputs. The outcome of this integration is then projected to higher cortical regions where the learned and emotional information is added to adapt the behavioral outcome [31]. Interestingly, the feeding state has some influences on the orbitofrontal cortex and on the lateral hypothalamus, which indicates that the hunger or satiation status can influence the taste of food; just as one example, hyperglycemic rats experience a loss in sensitivity of glucose sensing neurons [32].

Another aspect which is integrated with the taste impression of food, is the prior experience that is associated with the specific taste. Even low-level organisms like snails or mollusks developed various possibilities to sense nutrients in their environment and furthermore were able to associate these impressions with painful experiences [33]. Similar is true for higher organisms. In other words, individuals typically try to avoid food of a specific taste if this taste has been associated with a disturbance of the individual's well-being. This phenomenon is used in conditioned taste aversion tests in different scientific approaches. Here, a neutral taste is basically paired with a negative stimulus in a classical conditioning set up. Afterwards, the formerly neutral stimulus becomes a conditioned stimulus and, if it has been associated with a negative quality, is avoided.

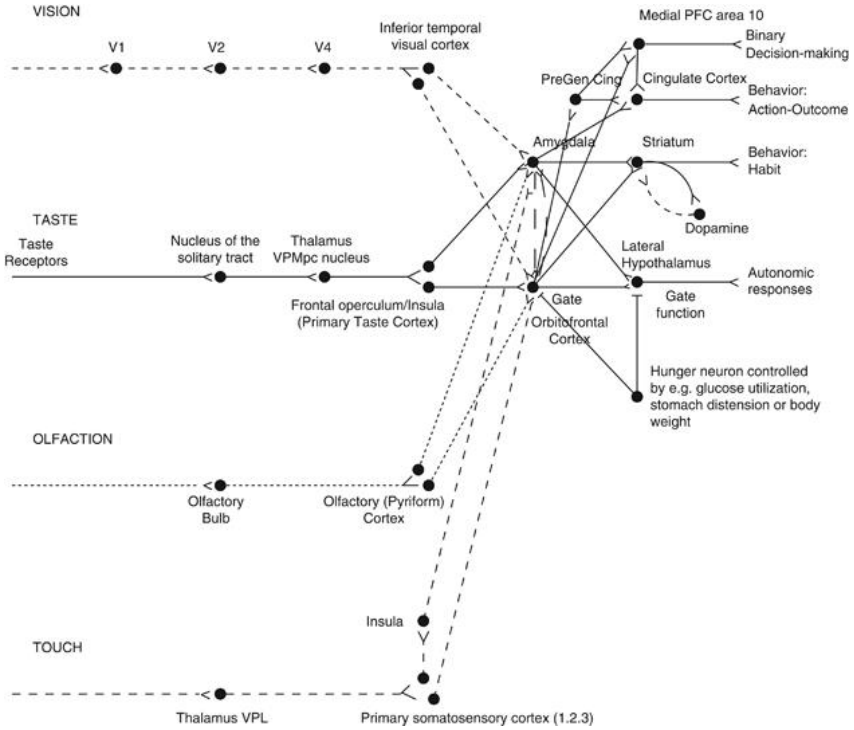

Figure 1-2: Schematic summery of the different somatosensory pathways projecting to the olfactory cortex (OFC) and some possible outputs. Gate refers to the fact, that some inputs evoke only effects when the right motivation state is reached e.g. hunger [31].

1.1.4 Fat as a separate taste sensation

Both rats [34] and mice [35] show a strong preference for high lipid solutions. This preference is consistent even if olfactory, textural and post-ingestive effects are excluded or minimized [36] [37]. Concerning the mechanism of fat sensing, several studies indicate that long chain fatty acids are somehow measured in the oral cavity of rodents [37, 38]. Initially, this seems puzzling because dietary fat is usually ingested in the form of triglycerides. However, because rodents have a very active lingual lipase and because inhibition of this lipase causes a tremendous loss in fat sensitivity [39], the model of sensing oral fat through long fatty acid receptors, like CD36 seems very plausible for rodents [40]. In comparison, humans have not such efficient lingual lipases [41]. Nevertheless, healthy adults are able to detect long chain fatty acids, even if olfactory and somatosensory inputs are minimized [42]. As typical dietary fatty food contains up to 0.5% long chain fatty acids [43], it is possible that these small amounts are sufficient and may be directly measured by a sensitive receptor without increasing the fatty acids from triglyceride breakdown by the lingual lipase reaction.

1.2 The obese state

1.2.1 Obesity treatment – bariatric surgery

There are various approaches to treat overweight and its associated risk factors. The traditional weight loosing strategies consists of a healthy diet in combination with increased physical activity. It may provide a short-term weight loss of up to 7% [44], but usually fails to enable the patients to maintain their reduced body weight over longtime periods [45]. Another traditional approach uses various therapeutics; e.g. central acting drugs like diethylproprion, fenproporex, mazindol showed satisfactory weight reducing effects [16]. Nevertheless, even with the most successful therapeutics, the maximum body weight reduction is 10%. [46].

The most invasive, but also most effective obesity treatment is bariatric surgery. Depending on the exact surgical procedure, body weight loss of up to 35% is achieved [47] and can often be maintained over long time periods [48]. From the different bariatric surgeries which include the gastric banding or sleeve gastrectomy, the Roux-en-Y gastric bypass (gastric bypass) is referred to as the gold standard. The first report of a gastric bypass was by Mason and Ito in 1966 [49]. Griffen first published the Roux-en-Y gastric bypass, which is commonly used today, in 1977 [50]. During a gastric bypass surgery, a small gastric pouch is created and connected to more distal segments of the small intestine. The respective limb is called the Roux-limb which combines with the biliopancreatic limb to form the common channel. By altering the length of the Roux-limb and the size of the gastric pouch, the weight loss effect can be tuned [51].

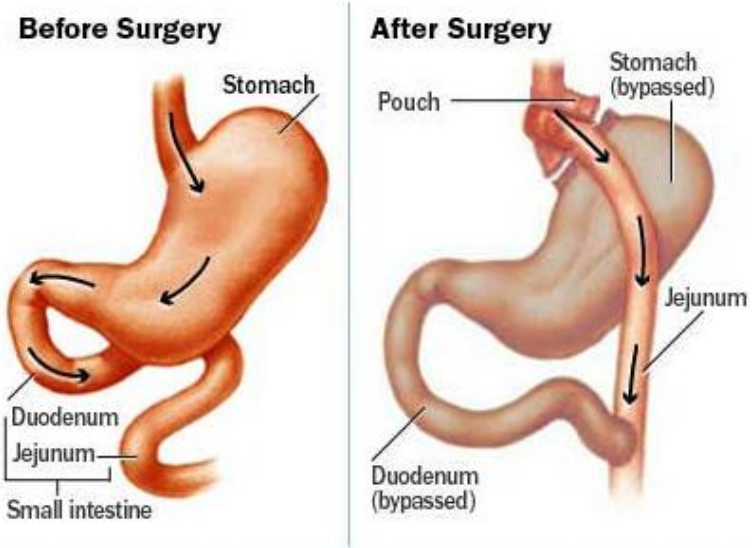

Figure 1-3: Comparison between the different passages of the stomach and the proximal part of the small intestine under physiological and artificial Roux-en-Y gastric bypass situation. http://www.mayoclinic.com/

Gastric bypass surgery leads to a number of changes resulting in a reduction in body weight. Humoral changes that influence appetite, eating behavior, glucose homeostasis and lipid metabolism are in the major focus of research, but the causal contribution of such changes to the effects of gastric bypass surgery is not always clear [51]. Because of the important endocrine component, the Roux-en-Y gastric bypass is often referred to as a metabolic surgery, even if the exact mechanisms are still to be elucidated [52].

1.2.2 Improvements after RYGB in patients

The most important reported effect of gastric bypass is of course the reduction of body weight [53] [54], which is most pronounced in a loss of fat mass. Patients undergoing gastric bypass loose up to 50% of total body fat with the biggest reduction seen in visceral fat [55]. Reduced body weight and body fat is due to different effects. First food intake in general is decreased, involving a reduction in meal size and meal frequency [56]. Furthermore energy expenditure is elevated, even though body weight is decreased [57]. This is striking because usually energy expenditure decreases as a compensatory mechanism during weight loss, e.g. when weight loss has been achieved by dieting [58]. Third, the food preference is also changed. This is remarkable because reduced eating after bariatric surgery has often been claimed to be due to a mechanical restriction linked to the small gastric pouch. If this were so, one would rather expect an increase in the intake of calorically dense food items in an attempt to overcome the caloric deficit imposed by the restrictive component; obviously, this is not the case. It has been shown that the food preference for both sweet and fatty food items is diminished after gastric bypass surgery. Patients reported that such food loses its attractiveness, which implies that somehow the rewarding system may be altered [59]. Underlining this hypothesis it has been shown that gastric bypass alters the neuronal response to high fat food in a functional magnetic resonance study [60].

Beside the massive reduction of body weight, a striking effect of gastric bypass in morbidly obese patients (BMI > 35) is the resolution of type two diabetes mellitus [61] and many other obesity-related comorbidities. Finally, many gastric bypass induced effects are long lasting and may be maintained over many years. This has been shown in different follow up studies,

like e.g. Valezia et al published in 2011, who investigated the body weight reduction eight years after gastric bypass [62].

Finally, an interesting, but less studied feature of bypass surgery is the alteration of bone quality. Bone turnover seems to be elevated after Roux-en-Y, while bone density decreases [63]. On one hand it has been claimed that this phenomenon can be explained by a reduction in body weight, which in turn reduces bone density, because bone structure is also a result of the mechanical forces [64]. Another explanation is, that not only gastrointestinal hormone levels are changed by bypass surgery, but also thyroid hormones and PTH [65]. PTH is responsible for the formation of bone mass and because bone is constantly rearranged, changes in PTH levels may have a deep impact on bone density [66].

1.2.3 RYGB in rats

The Roux-en-Y rat model that is established in our laboratory shows many similarities to what is observed in human patients [67]. In this model, both food intake and body weight are decreased dramatically after surgery [68], and the reduction of body weight is achieved mainly by a decrease in body fat [69]. The changes of total food intake are based on a reduction in meal size, while other meal parameters may in fact be elevated, e.g. meal frequency [69]. Additionally, the energy expenditure is elevated andenhances the weight loss [70] [71]. The increased energy expenditure indicates a complex alteration of the physiology after bariatric surgery. The same may be true for reported changes in food rewarding after bypass surgery [72]. It has been shown that both the intake of Ensure, a high-energy liquid, and of high fat chow are decreased after surgery in comparison to sham-operated rats [69]. This observation was true for lean and overweight rats and was not gender specific [73]. Furthermore this effect was not fat selective, because the positive responses to sugar were also decreased [74] [75].

Many of the various metabolic changes after gastric bypass may involve changes in the communication between central circuits, the peripheral gastrointestinal tract and fat depots. In fact, gastric bypass alters the endocrine status and different hormones have been investigated for their role in mediating the effects of bariatric surgery. Most studies reported elevated PYY [76] [75] [77] and GLP-1 levels, at least when measured post-prandially [75] [77]. Ghrelin plasma concentrations, on the other hand, are decreased[78]. CCK function is still discussed controversially. In some studies no CCK mediated effect was seen, whereas others showed

that there are differences in CCK concentration between control subjects, and patients with high and lower weight reduction after gastric bypass [79] [80] [81]. Another circuit that is influenced by gastric bypass is insulin action. Diet induced obese rats are often normo-glycemic with low insulin levels after surgery, and have a lower glucose to insulin ratio [82]; hence their insulin sensitivity is improved.

1.3 Approaches and Hypothesis

Morbid obesity has an alarming increase in prevalence over the last decades. Because gastric bypass surgery can be considered as the gold standard therapy, the scientific interest in mechanisms mediating the effects of bypass surgery has increased in parallel. The major aim of this study was to further investigate the observed shift in food preference as well as the endocrine mechanisms underyling changes in bone metabolism after gastric bypass operations in rats.

1.3.1 Brief access test

It is known that both patients and rats have a lower preference for high fat food after gastric bypass surgery. The question was whether this shift in food choice was due to altered taste qualities. Therefore, rats were tested in a Davis Rig's device, which enables the study of taste directed responses in rats while excluding post absorptive effects on food choice; the experimental approach is also called the brief access test.

1.3.2 Conditioned taste aversion tests

In the conditioned taste aversion test, the hypothesis was tested whether reduced fat preference was due to the induction of visceral illness in bypass rats after ingesting a standardized fat load. Therefore fat taste was paired with a LiCl injection which evokes strong visceral pain. After three repetitions, those rats which paired the taste of fat with the feeling of sickness were expected to develop a very strong taste aversion. The fat acceptance of these rats was than compared to food choice of bypass and sham rats, which received only NaCl injections.

1.3.3 Two bottle preference test

In the two bottle preference test, the fat intake in the form of an Intralipid© solution was measured over 48 hours and therefore included both spontaneous taste and downstream post-absorptive effects. Results of the first and second dark-light cycle were compared to see whether the spontaneous taste choice or the post absorptive effects are more important. If it were the latter, fat intake would be expected to differ between both exposures because absorption takes time, while spontaneous taste cues are quite fast and therefore should already be present on first presentation.

1.3.4 Hormone measurement

From both human and animal trials, it is well known that several gastrointestinal hormone concentrations differ after bypass surgery. To investigate whether our rat model showed similar effects, plasma hormone levels were measured from short term satiation (GIP, GLP-1, amylin, PP and PYY) and long term adiposity signals (insulin and leptin).

1.3.5 Refeeding experiment

The refeeding experiment aimed to investigate if gastric bypass rats can ingest large meals under appropriate conditions; in other words, we wanted to test whether meal size is decreased after gastric bypass because of a mechanical limitation due to the small stomach pouch. Therefore, rats were restricted to 50% of usual food intake for three days and the refeeding response was then measured. In a second trial, different fasting intervals were tested, to verify if the three-day fasting protocol was sufficient to create sufficient drive for subsequent overeating.

1.3.6 Bone density measurement

Bone density measurements were conducted to test whether known structural alterations in bones after gastric bypass are due to a decrease in body weight or if there are more complex mechanisms directly related to the type of surgery. Therefore, bone density of bypass rats was compared to sham operated rats and to sham operated rats that were body weight matched to rats that received gastric bypass surgery.

2 Rats, Materials and Methods

2.1 Rats and housing conditions

For all experiments male Wistar rats were used (Elevage Janvier, France) . The rats were individually housed in wire mesh floor cages to prevent contamination of the wounds after surgery and to enable us to measure individual food and water intake. Food and water were available ad libitum, except where noted otherwise. The light cycle was fixed to a 12h/12h dark-light pattern, with the dark onset at 13.00. While the rats were housed in the metabolic cages (see below), they were kept in single plastic cages with wooden shaving.

2.2 Metabolic cages (PhysioScan System)

The PhysioScan Systems were used to analyze meal patterns during refeeding periods. Besides the recording of food and water uptake, this system provides the additional function of gas exchange measurement by an open circuit calorimetry system (*AccuScan Instruments, USA*), but this function was not used in our experiments.

2.3 Food and water consumption

Rats housed in single wire mesh cages received two bottles of water which were checked daily, and standard chow pellets (*3433; Provimi Kliba AG, Kaiseraugst, Switzerland*). For the experiments in the metabolic cages, the rats received food of an equivalent composition in powder form, which prevents food hoarding (*3436; Provimi Kliba AG, Kaiseraugst, Switzerland)* and a single water bottle. The water bottle and the food cup were placed on sensitive scales for monitoring the eating and drinking process. Food intake and water intake were measured continuously.

Dry matter	88%
Protein	18.5%
Fiber	4.5%
Fat	4.5%
Ash	6.3%
Nitrogen-free extract (NFE)	54.2%
Starch	35.0%
Metabolisable energy	13.2 MJ/kg

Table 2-1: Nutrient composition of pellet and powder standard chow diets (*Provimi Kliba AG #3433 and #3436*)

2.4 Chemical compounds

2.4.1 Salts for taste aversion tests

For conditioning the taste aversion against Intralipid©, 0.3 M lithium chlorid solution was used. 0.3 M sodium chloride was used as control. Both salts were ordered from *Sigma-Aldrich Co* and diluted in distilled water prior to injection.

2.4.2 Intralipid©

Intralipid© (20 %) was ordered from *Fresenius Kabi (CH, AG, 6371 Stans)* and diluted with water to the respective concentrations. The active agent of Intralipid© is purified soybean oil, which is enriched with phospholipids from eggs and glycerol to enhance the water solubility; Intralipid© can be diluted with water to any desired concentration.

2.5 Surgery and laboratory procedures

2.5.1 RYGB Surgery

Prior to surgery all rats were fasted overnight (12h), but had constant access to water. The rats were weighted and then received i.p. 10 mg/kg of the antibiotic agent Enrofloxacin (0.4 ml/kg of 2.5% Baytril®, Bayer Health Care; Provet; Lyssach), 5 mg/kg of an analgesic and anti-inflammatory agent (Finadyn®, Essex Tierarznei Provet; Lyssach) and saline. Afterwards they were put into the induction chamber, with 5% isoflurane (*IsoFlo®; Provet AG, Lyssach, Switzerland*). After reaching a surgery-tolerant anesthesia level, the rats were removed from the induction chamber and switched to nose cone anesthesia with 2.5% to 3% isoflurane. The surgical site was shaved and disinfected (*Betadine®; Provet AG*), and the eyes of the rats were protected from drying out with vitamin A ointment. During surgery the rats were placed on heating pads to avoid dropping of body temperature.

To create the biliopancreatic limb in gastric bypass rats, the proximal jejunum was cut 10 cm distal to the pylorus. After localizing the caecum, the ileum was joined with a 7 mm side-to-side jejuno-jejunostomy (running prolene 7-0 suture) which lead to a 25 cm long common channel. To mimic these interventions and the surgical stress in sham rats, a 7 mm long gastrostomy in the anterior wall of the stomach with a subsequent closure (interrupted prolene 5-0) and a 7 mm long jejunostomy with subsequent closure (running prolenen 6-0) was performed. After closing the abdomen, all rats received one single dose (0.1 ml/kg) of an opioid analgesic agent *(Temgesic®; 0.3 mg/ml; Reckitt Benckiser, Wallisellen AG)* and were placed under red light during the awaking phase. All rats were treated with Baytril and Finadyn, with the doses described before on the first and second postoperative days.

2.5.2 Lickometer training and testing

The Davis Rig was developed in the 1990 at The Florida State University [83] and is here referred to as Lickometer. The Lickometer allows the study of taste directed reactions and minimizes post-ingestive effects due to the small volumes swallowed in single tests. The Lickometer consists of a plastic cage with wire mesh floor and one drinking opening. This opening is closed by a flexible shutter, which is connected to a small motor device. In front of the drinking place is a movable holder, which has space for 16 drinking flasks. Both, shutter and the rig are connected to a PC program

that synchronizes the movements. If the rat licks on the bottle sprout, the system registers the number of licks. Due to that, it is possible to measure both, quality and quantity of licking behavior of 16 different liquids in one set up.

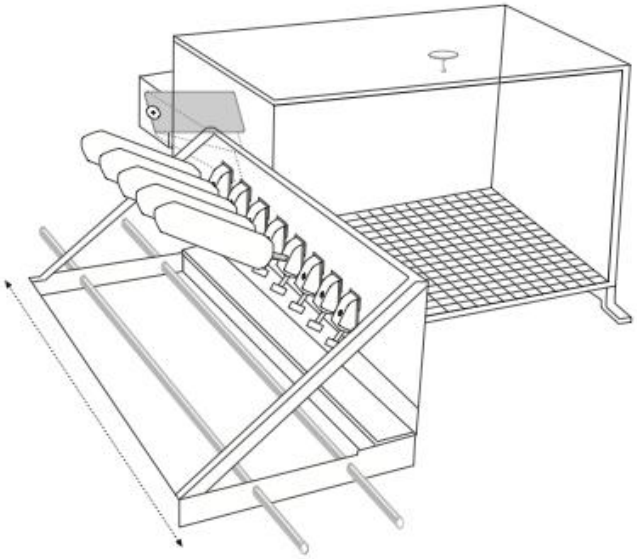

Figure 2-1: The Davis Rig or Lickometer with cage, bottle holder (here for 8 flaks) and shutter [83].

To habituate the rats to the unfamiliar Lickometer and the movement of the Davis Rig and the shutter, the rats had to be trained in the Lickometer prior to the experiments. For training sessions, eight flasks were filled with water. The rats were water deprived for 24 h and then placed in the Lickometer for 30 min. The flask presentation followed the same program as later in the experiments. All eight flasks were presented in randomized order. After the eighth flask, the presentation started again in a new randomized order. If the animal did not initiate a trial by licking, the shutter stayed open for 60 sec, until it closed and moved on to the next flask. If the animal initiated the trial, it had access to the flaks for 10 sec only. For the proper experiments, the flask were filled with seven different concentrations of Intralipid© and water, according to Table 2-2.

Flask	1	2	3	4	5	6	7	8
Intralipid© (%)	0, water	0.005	0.01	0.05	0.1	0.5	1	5

Table 2-2: Concentrations of Intralipid© that were tested during experimental trials

Beside that, the experiments were not different from trainings sessions; however, the rats were tested under different food and water restriction conditions and not always tested after 24 h deprivation.

2.5.3 Conditioning of taste aversion against Intralipid©

To test whether gastric bypass rats develop a taste aversion against Intralipid© in comparison to sham rats, we needed adequate control rats to define aversive reactions. Therefore, one group of sham rats and one group of bypass rats were treated with LiCl injections after receiving an oral bolus of Intralipid©. LiCl triggers toxic effects and is used since many years as a positive control in taste aversion tests [84]. By giving the LiCl after the oral Intralipid© stimulus, the rats associate the following nausea with Intralipid© and will avoid this specific taste afterwards.

Sham and bypass rats were devided in two groups, one testing group and one aversion control group. After fasting the rats for 6 h, they received 1 ml of 5 % Intralipid© p.o. and received an i.p injection of either NaCl or LiCl (76 mg/kg) 20 min later. This conditioning paradigm was repeated on three days with 48 h recovery intervals in between.

2.5.4 Two Bottle Test

For the two bottle preferences test, all rats received one bottle filled with water and one filled with 5 % Intralipid© solution. The rats had free access to food while the solutions were offered ad libitum for 48 h. The bottles were weighed at the beginning, after 24 h and after 48 h. To control for spillage of water or Intralipid© due to removing the bottles from the cage, a separate water and Intralipid© filled bottle were fixed at an empty cage, and weighted along with the testing bottles; all volumes were corrected for spillage.

As the rats of the two bottle test were already used for the Brief Access Test, the animals were not Intralipid naiv at the time point of the two bottle testing.

2.5.5 Blood and tissue samples collection

Blood was sampled at the termination of the experiments. The rats were fasted for six hours. After decapitation, blood was collected directly from the opened aorta. A GLP-1 degradation inhibitor *(DDP IV Inhibitor, Millipore, USA)* and aprotinin (*Aprotinin, P2714 Protease Inhibitory Cocktail, Sigma Aldrich, Basel, Switzerland*) were added to the blood samples to stop the degradation of the peptide hormones and particular GLP-1. Plasma was obtained by centrifugation and stored at -80 °C. One hind leg was frozen at -80 °C; for the analysis of bone quality, all muscle tissue was removed.

2.6 Statistical analyses

All data are represented as mean ± SEM. The data were tested for normal distribution with a Komogorov-Smirnov test, and statistical significance was tested using two-way ANOVA and Bonferroni's post-hoc comparison. A P-value < 0.05 was considered statistically significant. The software in use was Prism Version 5.0a for Mac OS X (*GraphPad Software Inc., San Diego, CA, USA).*

2.7 Experimental design

2.7.1 Brief access test

In the brief access test, three separate groups of rats were tested. The first group consisted of seven sham operated rats, the second of four bypass rats and the third group of eight sham operated rats with their body weight matched to bypass rats. This was done by food restricting these rats to about 50 % of their voluntary intake directly after the recovery period, one-week post surgery. At the beginning of week 19 post surgery, all rats were trained in the Lickometer, as described above. After two days of recovery from the water deprivation, all rats were tested under ad libitum and water deprived condition (deprivation time 24 h). In between, 48h recovery periods were given.

2.7.2 Conditioned taste aversion test

Four animal groups were tested. Ten sham operated and seven bypass rats were divided into positive aversion groups, which were trained with LiCl injections after being given Intralipid© orally, and testing groups that received NaCl injections instead of LiCl. In the 16th week post surgery, all rats were trained in the Lickometer as described above. Beginning after a 72 h recovery period, the rats received one bolus of 1 ml Intralipid© 5% orally and LiCl or NaCl 20 min later, respectively. This bolus was given directly on the back of the tongue, without using gavage.

This procedurewas repeated three times with 48h recovery in between. After an additional 72 h recovery period, the rats had a second Lickometer training and were finally tested in weeks 17 and 18 post surgery. Three different feeding states were tested as described for the brief access test.

2.7.3 Two bottle preference test

The two-bottle test was done in week 20 post surgery. Rats used before in the brief access tests and the conditioned taste aversion tests were included (14 sham and 5 bypass rats from the brief access tests; 10 sham rats and 4 bypass from the aversion test). As described above, all rats received bottles filled with water or Intralipid© (5%) in their home cages, to which they had access ad libitum. Fluid ingestion was measured after 24h and 48h.

2.7.4 Blood hormone measurement

The hormones, amylin, insulin, glucagon, PP, GLP-1 and leptin, were measured with a hormone kit (LINCOplex Mouse endocrine, Millipore, USA). This kit used a conventional sandwich assay technology and provided the detection of several hormones in parallel. The plate was analyzed using the Bio Plex Suspension Array System (*Bio-Rad Laboratories, Rheinach; BL, Switzerland*).

2.7.5 Refeeding experiment

The rats were placed in the metabolic cages which allowed the continuous measurement of food and water consumption. To test the rats' ability to compensate for prior food deprivation in a refeeding period, 16 unoperated rats were placed in the

metabolic cages. After four days to get used to the new surrounding, the restriction period was initiated. One group of 5 rats was food restricted by 50 % for 14 days, one for 3 days and the last group was not fasted at all. The refeeding started at dark onset, simultaneously for all groups. The food and water consumption were recorded for three dark night cycles following the end of the restriction period. In a second experiment, 5 sham and 7 bypass rats were placed in the metabolic cages. After a 4 day acclimatization, all rats were restricted for three days. The refeeding was done as described before.

2.7.6 Bone density measurement

After decapitation of the rats at study end, one hind leg of each animal was amputated and frozen at -80 °C. Later the muscle layers were removed and the blank femur was send to A. Liesgang and colleagues (Institute of Animal Nutrition, UZH), who analyzed the bone density by CT scanning as described by Abegg and colleagues in 2013 [85] .

3 Results

3.1 Postoperative recovery

After gastric bypass or sham surgery, respectively, all rats were given at least six days for recovery. Thereafter, the sham groups already started to gain weight, while bypass rats lost body weight up to six days post surgery (Figure 3-1). Of note, food restriction in the sham operated rats body weight matched to the bypass rats started only on day eighteen after surgery.

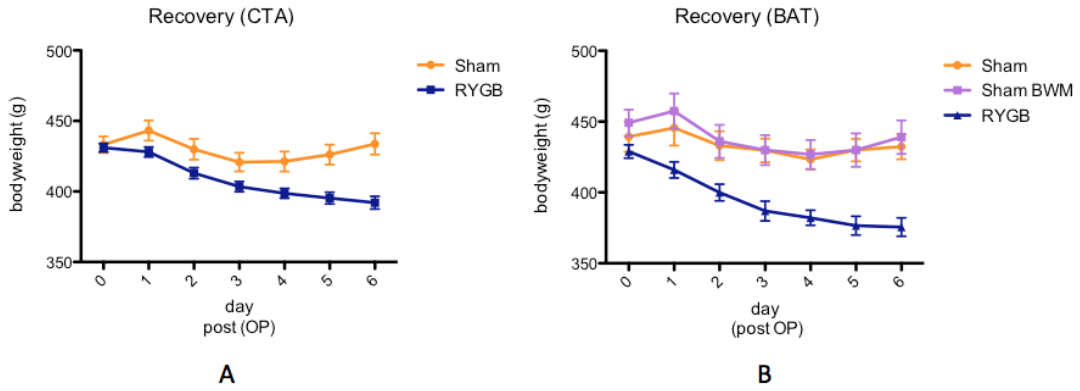

Figure 3-1: Body weight gain in the immediate post-surgical period after sham or bypass surgery on day 0. *A* shows the development of rats used for conditioned taste aversion tests (CTA) and *B* for brief access tests (BAT). In both cohorts, bypass rats lost weight up to post surgery day six while sham operated rats were already stable and started to gain weight (n=8)

After the recovery period, sham and bypass rats were fed ad libitum. The body weight matched rats were food restricted to about 50% and received therefore about 15g chow per day. In the conditioned taste aversion rats, bypass rats had a significantly lower body weight two weeks after surgery. The same was true for the rats used in the brief access tests; the body weight matched rats which had started with a similar body weight as ad libitum fed sham rats reached the average body weight of bypass rats in week three (Figure 3-2).

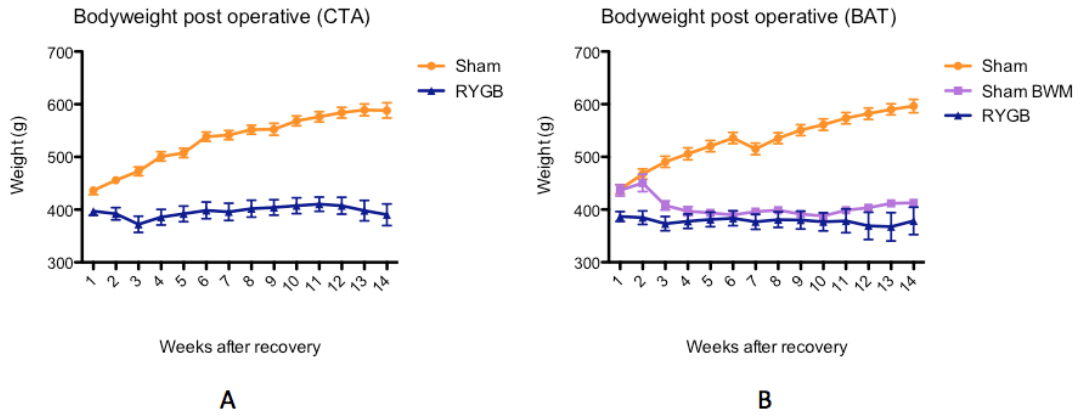

Figure 3-2: Body weight gain over all experimental weeks, starting after the immediate recovery period. Bypass rats had significantly lower body weight, over the complete period. Body weight matched rats were put on a restriction paradigm and reached the body weight of bypass rats in week three (n=8, exept for CTA sham n=12).

3.2 Brief access test

Two different conditions were tested, i.e. after ad libitum access to food and water, and after 24 h water deprivation. The data are shown as absolute licks and as the number of licks relative to water (Figure 3-3). Under ad libitum conditions, there were no differences between sham and bypass rats. Both groups prefered higher Intralipid© concentrations ($p < 0.0001$).

In the water-deprived trials, both bypass and sham rats always licked at maximal speed, even when water was presented in the Lickometer. Therefore no concentration dependent selection was observed and the calculated relative data were clustered around zero.

Under the water-deprived condition, the body weight matched rats were also tested. These rats had not been tested in the ad libitum trails, because they were under constant restriction to keep the required body weight. Interestingly, the body weight matched rats significantly preferred Intralipid© concentrations of 0.5 % and higher relative to water. It is important to note, however, that all three groups of rats showed a similar licking response at high Intralipid© concentrations.

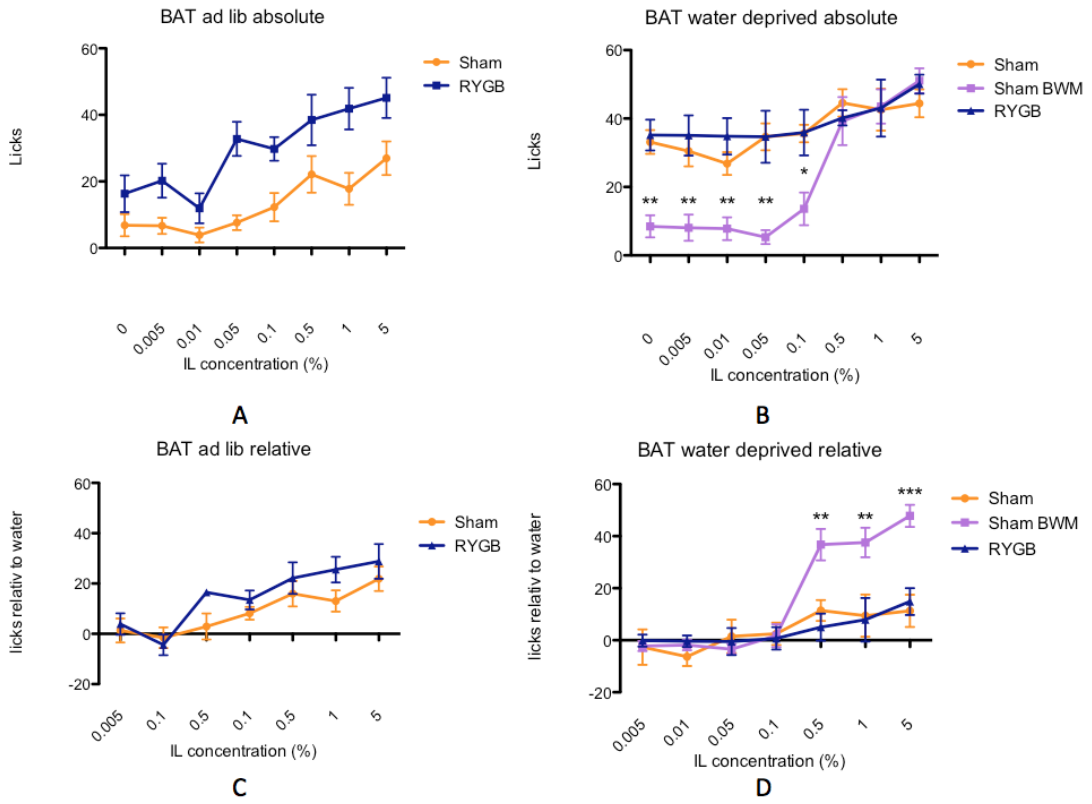

Figure 3-3: Licking responses shown in absolute licks (A, B) and licks relative to water (C, D) under ad libitum (A, C) and water deprived (B, D) conditions. Significant p-values less than 0.05 are marked with one, such less 0.01 with two and such less than 0.001 with three stars. After water deprivation, sham and bypass rats showed no concentration dependent selection, i.e. in contrast to what was visible under ad libitum conditions. The food restricted body weight matched rats preferred concentrations of 0.5 % Intralipid© and higher). However, absolute licking responses did not differ at high concentrations (n=8).

3.3 Conditioned taste aversion

The data are shown in relative and absolute licks in both tested situations, i.e. ad libitum and after 24 h water deprivation (Figure 3-4).

Under ad libitum conditions, the naive Intralipid© sham rats significantly preferred Intralipid© concentrations of 0.1% and higher relative to water. The other three groups had low licking responses, irrespective of which tube was presented. In other words, the sham operated LiCl treated rats showed the expected reduction in

Intralipid© intake; further, the Roux-en-Y gastric bypass rats that received NaCl had an Intralipid© preference that was as low as after being conditioned with LiCl.

Under water deprived conditions, both LiCl injected groups had significantly lower licking rates at tubes filled with 0.05% Intralipid© and higher, when plotted as absolute licking responses. Due to the fact that the standard deviation was relatively high and that both saline injected groups had high licking responses even when water was presented, no significant differences were seen when licking was expressed relative to water.

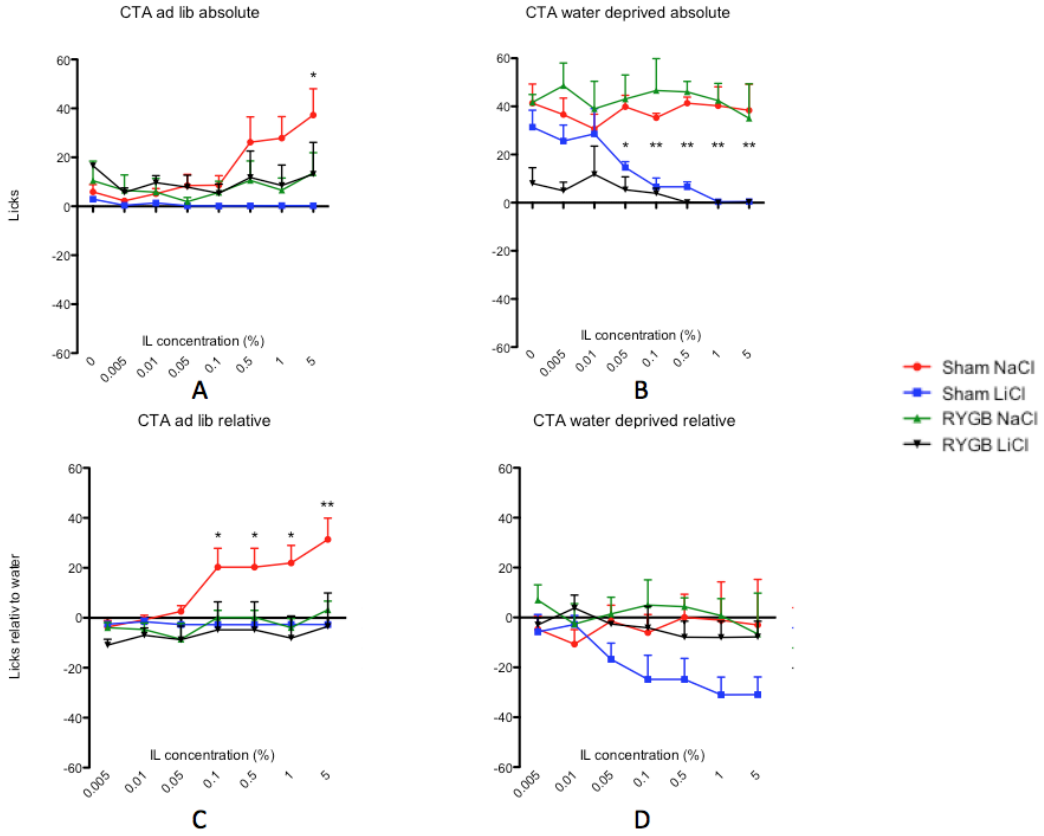

Figure 3-4: Licking responses of taste aversion test shown in absolute licks (A, B) and licks relative to water (C, D) und ad libitum (A, C) and water deprived conditions (B, D). Intralipid© naive rats had a significantly higher relative Intralipid© uptake of 0.1% Intralipid© concentration and higher, in comparison to all other groups. Under water deprived conditions, both LiCl injected groups had lower absolute licking responses if 0.05% Intralipid© or higher concentrations were presented. Due to the group dependent responses to water, these differences were not visible when expressed as relative licking responses (n=5, except for RYGB n=3).

3.4 Two bottle preference test

Data are shown in absolute volume in ml Intralipid© and as intake relative to total fluid intake (Figure 3-5).

Rats. It was found that body weight matched sham rats drank significantly more Intralipid© over the first time interval when compared to gastric bypass rats. On the other hand, gastric bypass rats drank significantly less Intralipid© than sham rats. Due to the higher total intake in the body weight matched group, no differences in Intralipid© intake were seen between body weight matched and sham rats when expressed in relative values. In the bypass rats, the reduced Intralipid© intake over the second time interval (24-48h) was consistent with our expectation.

In rats that had been used in the conditioned taste aversion test before, both groups previously being treated with LiCl drank less Intralipid© than the NaCl treated groups, irrespective of the time period and irrespective whether absolute or relative data were considered. Importantly, saline injected bypass rats drank significantly less Intralipid© than saline injected sham rats.

The higher absolute intake of Intralipid© in the sham group during the second interval in comparison to the first 24h was paralleled by a higher overall liquid intake because this difference was no longer visible in the relative dataset.

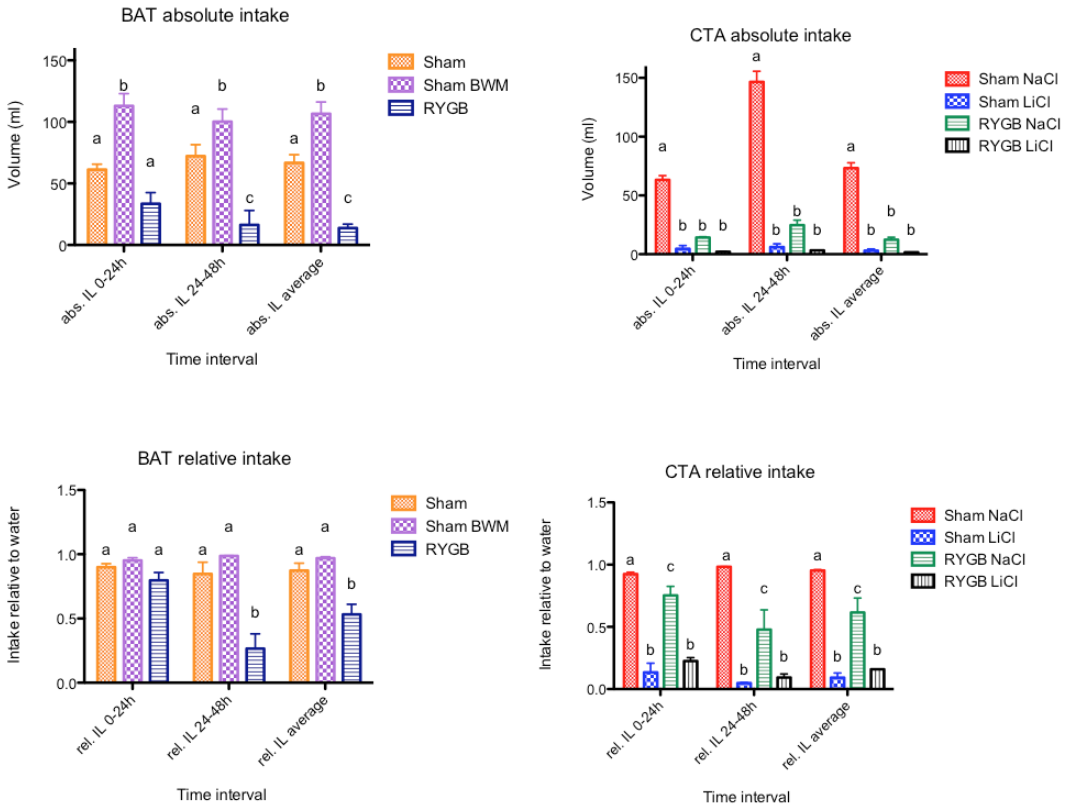

Figure 3-5: Intralipid© and water intake after 24 and after 48 hours in the 2-bottle test, shown for the rats that had been used before in the brief access test (A, C) or the conditioned taste aversion test (B, D) before; data are presented as absolute volume of Intralipid© ingestion (A, B) and as relative volumes compared to water (C, D). In the brief access rats, body weight matched rats drank more Intralipid© than sham rats in the first 24 h interval, while bypass rats drank less. In respect to relative intake, sham and body weight matched rats drank the same amount, while bypass rats had a reduced relative Intralipid© intake in the second interval. In the conditioned taste aversion test rats, both previously LiCl injected groups drank less than the saline groups, concerning relative intake. For absolute values the elevated Intralipid© intake of saline sham rats is consistent with our expectation.

Group sizes were in BAT: Sham=8, Sham BWM=6, RYGB=5 and in

CTA: Sham NaCl=6, Sham LiCl=4, RYGB NaCl=2, RYGB LiCl=2.

3.5 Hormone measurement

The results are shown in Figure 3-6. For PP, GIP and insulin, no significant changes were seen, but bypass rats showed a significantly elevated level of plasma PYY. Furthermore, a nice gradient from significantly higher leptin values in sham rats to much lower levels in bypass rats or weight matched rats were visible. Interestingly, body weight matched rats had the same body weight as bypass rats but much higher leptin levels. GLP-1 was measured, too, but all values were below detection limit.

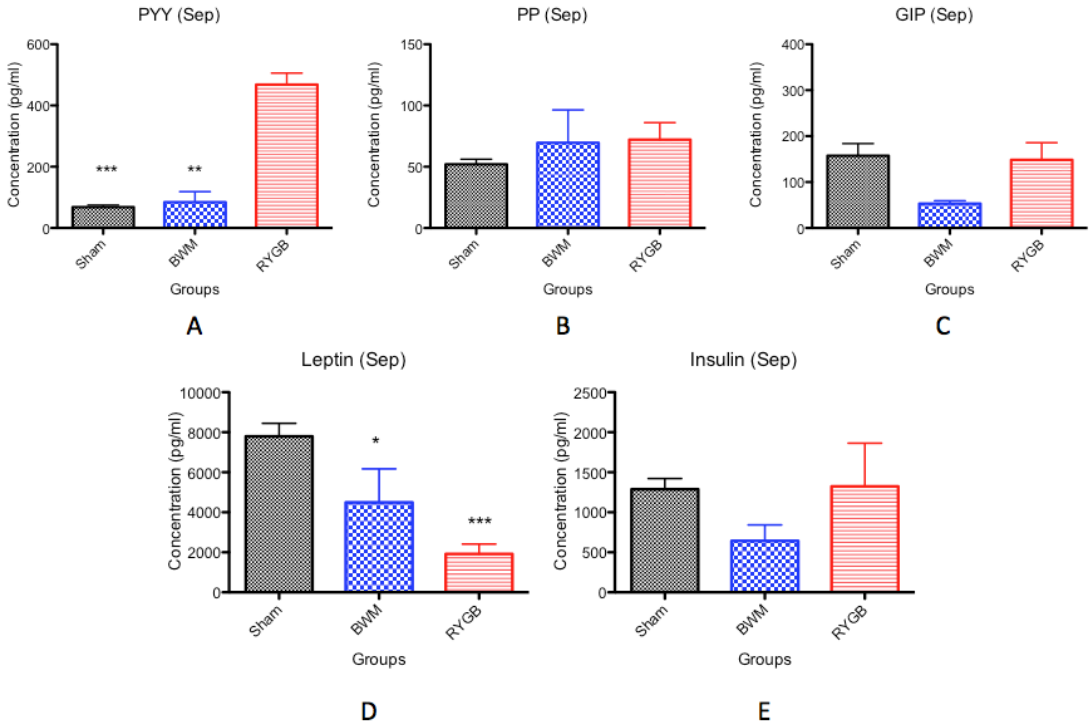

Figure 3-6: No significant changes in plasma levels were observable for PP, GIP and insulin. PYY was significantly elevated in bypass rats and leptin was higher in sham rats compared to body weight matched rats, which in turn were higher than in bypass rats. Twenty sham rats were sample and eight sham body weight matched, RYGB respectively.

3.6 Refeeding experiment

The analysis of the size of the first meal after termination of food restriction indicated that non-operated rats that had been restricted for 14 days ate twice as much as the other groups. Subsequent meals did not differ (Figure 3-7).

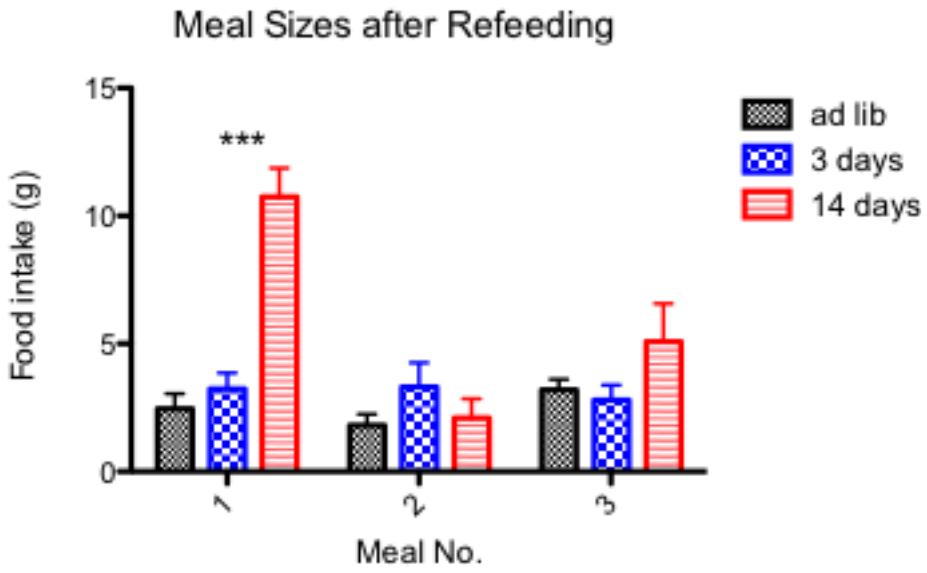

Figure 3-7: The sizes of the first three meals after refeeding are shown. The rats are grouped according to the duration of the prior restriction period of either none, three or fourteen days. The only significant difference was that rats ate twice as much after fourteen days of restriction in the first meal after refeeding.

In a second experiment, bypass (n=7) and sham rats (n= 5) were restricted to 50% for three days and then refed as described above. No differences were seen between sham and bypass rats (Figure 3-8). Furthermore both groups had similar food intakes as ad libitum and three days restricted rats in the previous experiment. While the first test indicated that extended food restriction may be necessary to see a clear response at the time of refeeding compared to ad libitum fed rats, we felt that it was inappropriate to food restrict Roux-en-Y gastric bypass rats for such a long period.

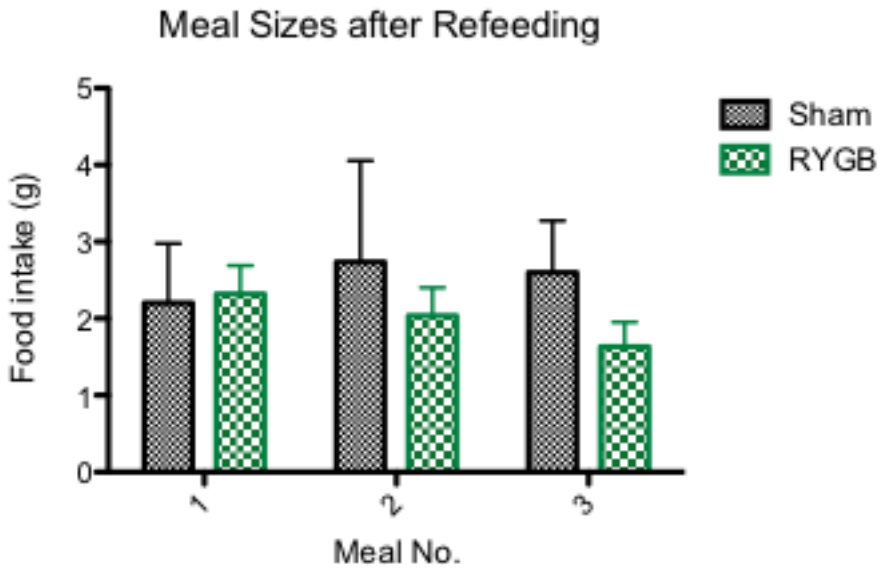

Figure 3-8: The size of the first three meals after refeeding of sham and bypass rats are shown; rats had been 50% food restricted for 3 days. No differences between both groups were seen.

3.7 Bone density measurement

The differences in bone density are shown in Figure 3-9. The bone length was the same for all groups, but Roux-en-Y gastric bypass rats had dramatic changes in all parameters of density, i.e. total, cortical and trabecular density. No difference was seen between sham and body weight matched rats.

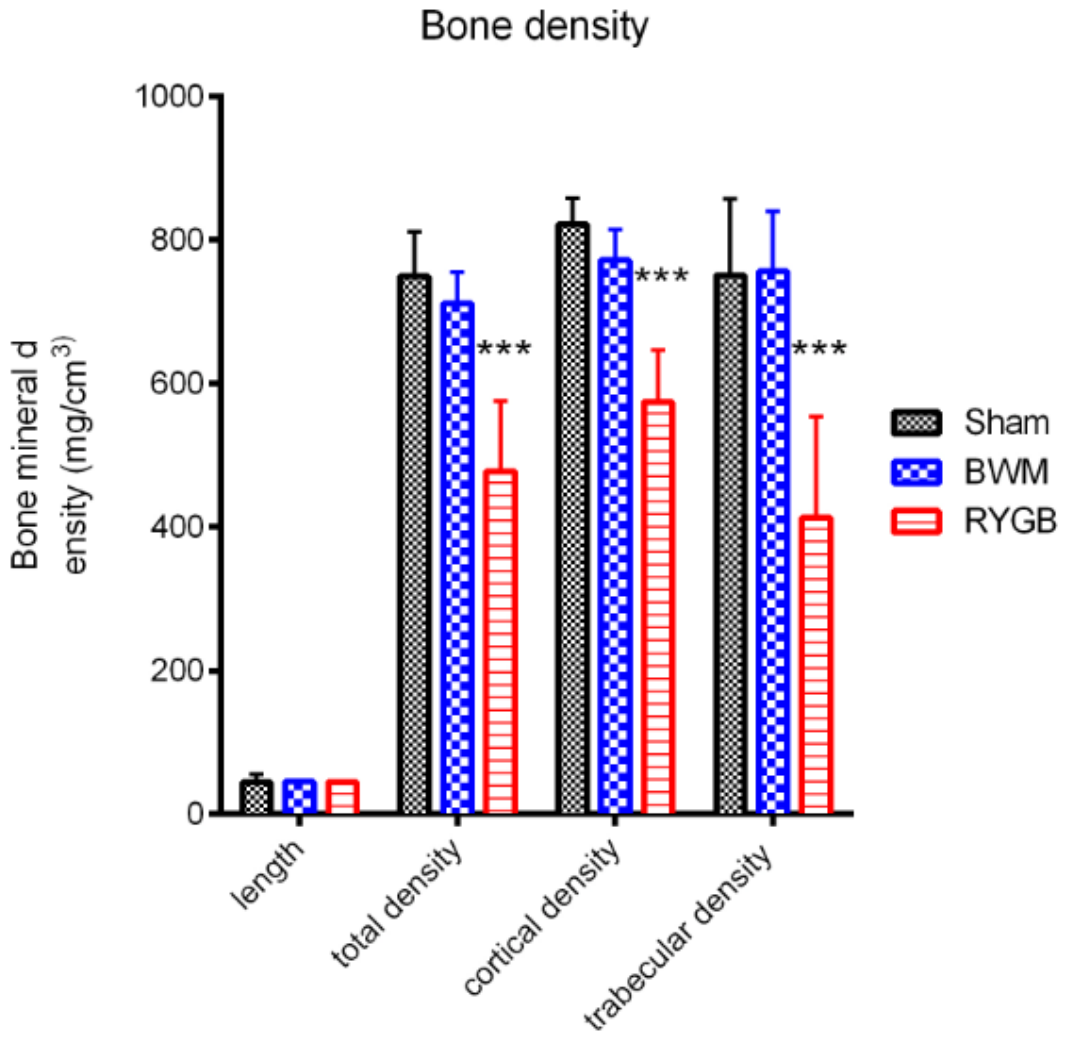

Figure 3-9: Fermur Ct scans showed a significant decrease of bone mass in total, cortical and trabecular density of bypass rats in comparison to both other groups, while bone length was the same.(BMD bone mineral density mg/cm3). Group sizes were Sham=18, Sham BWM=8, RYGB=8), bone length was measured in cm.

4 Discussion

4.1 Post operative recovery

Before surgery the rats were randomly separated into bypass or sham operated groups. After surgery, the bypass rats lost approximately 50 g and then stabilized their body weight about one week post surgery. Sham operated rats lost less weight than bypass rats after surgery and started to regain body weight around postoperative day 5. Until postoperative week 14, bypass rats did not show any weight gain, but maintained their post surgery weight over the entire observation time. The same was seen in the experiments done by Fenske at al in 2011 [86], whereas Stearns and colleagues showed that their bypass rats still gained some weight even though less than sham rats [87]. It needs to be mentioned that the rats included in our study underwent repeated deprivation intervals that were necessary for training and testing the rats in the Davis Rig. It is possible that bypass rats are more sensitive to such restriction periods because the absorption of at least some nutrients may be limiting bypass surgery [88]. On the other hand, the difference between our study and the study by Stearns may also be due to some differences in the surgical approach [89].

The rats of the body weight matched group were set to a feeding paradigm starting on day six post surgery; these rats reached the average body weight of bypass rats after about 2 weeks. Their body weight was kept low by limiting their daily food intake to about 15 g, which is approximately 54% of the spontaneous food intake in sham operated ad libitum fed rats. This is similar to what we had observed previously [90].

4.2 Brief access test

Intralipid© naïve rats were used for the brief access tests, i.e. Intralipid© was offered for the first time during the first Intralipid© trial in the Davis Rig Lickometer, after the rats had undergone the Davis Rig training days, for which only water was used. Under ad libitum conditions, both sham and bypass rats preferred higher Intralipid© concentrations with no differences between the surgical group. Body weight matched rats were not included in ad libitum trails, because their Intralipid© intake is always higher, due to their restricted access to solid food.

In the water-deprived state, both sham and bypass rats did not select for higher Intralipid© concentrations, but it needs to be noted that all rats exhibited maximal licking responses at all tubes; hence, potential differences between low and high Intralipid© concentrations may have been asked. Importantly, however, no differences between sham and bypass rats were observed. Only the (food restriced) body weight matched rats drank more Intralipid© at higher than at lower concentrations. However, at high concentrations, the Intralipid© intake was similar in all groups.

From these results, three conclusions can be drawn. First, bypass surgery does not alter fat response of naïve rats in the brief access test, as no differences were seen between bypass and sham responses. Second, naïve rats prefer concentrated Intralipid© under ad libitum conditions, but this preference can be overridden by increasing the drinking motivation e.g. through water restriction. Third, body weight matched rats under a strict feeding paradigm have a strong positive fat response even in a water deprived state. Probably this can be explained by the food restriction itself, because the sensation of hunger alters drinking behavior and aldosterone response in rats [91] [92].

Independently from the reaction of the body weight matched sham rats, the most important outcome of these experiments was that bypass surgery did not evoke a change in spontaneous Intralipid© intake in the brief access test in naïve rats. In other words, it seems unlikely that the oral taste sensation for fat is altered by gastric bypass surgery and other mechanisms may prevail to explain the reduced fat preference in free feeding or two bottle tests [93].

4.3 Conditioned taste aversion test

In comparison to the brief access test rats, the rats used for the conditioned taste aversion test were not Intralipid© naïve. Beside the training days in the Davis rig Lickometer, which were done only with water, these rats received an oral Intralipid© bolus and were afterwards injected with NaCl or LiCl, according to their respective experimental group.

Under ad libitum condition, the sham operated NaCl rats preferred high Intralipid© concentrations, starting their positive selection at 0.1 % of Intralipid©. Both LiCl

injected groups avoided Intralipid©, which can be seen in the absolute and relative licking responses. Interestingly, the NaCl injected bypass rats avoided high Intralipid© intake. Consequently, Intralipid©, at least in high concentrations, seems to have an aversive effect in bypass, but not in sham operated rats.

Concerning the results derived from water deprived rats, both LiCl groups showed significant avoidance of Intralipid©, while the NaCl injected sham rats showed the highest licking responses to all tubes, without any selection. Thus, they reacted similar to the naive rats in the brief access test, where the fat preference was also overridden by high drinking motivation. Strikingly, bypass rats injected with NaCl showed a similar licking response in the water deprived state, i.e. high licking rates at all concentrations.

In the conditioned taste aversion test, we saw a clear fat avoidance in the bypass group, i.e. different from the brief access tests described above. We therefore believe that the simple taste of fat has no aversive effect in naïve rats, but that fat taste becomes a trained negative stimulus and is avoided after repeated exposure [69]. This indicates the presence of fat specific downstream effects that may be associated with nausea in the bypass rats. This hypothesis explains, that naïve rats preferred high Intralipid© concentrations similar to sham operated rats, and that after several trainings bypass rats start to avoid it [94].

The water deprived experiment showed two facts, first that there was a positive selection for fat in the sham group that can be masked by a general drive to drink, and that the same is true for the negative dismissal of Intralipid© by bypass rats. In both groups the specific negative or positive associations with Intralipid© may not have been as strong as the general motivation to drink caused by fluid deprivation over 24 hours. On the other hand, it was clear that both LiCl injected groups still avoided Intralipid© solutions at higher concentrations even after water restriction; this indicates that the aversive effect of LiCl was much stronger than that of fat in bypass rats under our conditions.

4.4 Two bottle preference test

The two-bottle preference tests in the rats that had been used before in the brief access tests showed clearly the positive reaction of sham and body weight matched rats to Intralipid© over all time intervals. Both groups had high relative Intralipid© intakes,

which means that they nearly drank 100% of their total fluid ingestion as Intralipid©. Interestingly, bypass rats showed different Intralipid© responses in the first and second 24-hour time interval. During the first 24 hours, they behaved similar to sham operated rats and had a high preference for Intralipid©. In the second interval, however, Intralipid© intake was much decreased by approximately 75%, meaning that the rats now clearly preferred water as a fluid. This underlines the hypothesis that Intralipid© avoidance after bypass develops slowly and may be due to some later digestive or post-digestive components; in other words, the avoidance needs a certain time until it can be seen.

The same seemed to be true for the rats that had been used before in the conditioned taste aversion experiments. Here, differences between high Intralipid© selection by sham rats and reduced uptake in bypass rats were already visible in the first 24 hour interval. Because these rats were not Intralipid© naïve, we in fact had expected differences in Intralipid© selection from the beginning; in other words, these results strengthen the idea of a learned behavior, i.e. delayed development of aversion against high fat intake after gastric bypass.

Further, the previously LiCl injected rats showed the lowest Intralipid© intake. This underlines the idea that LiCl evoked pronounced and long-lasting aversive effects.

4.5 Hormone measurement

Concerning the hormone measurements, it was interesting to note that leptin levels were lower in bypass rats than in body weight matched rats. Because leptin correlates with body fat, it was not surprising that sham rats had the highest leptin levels. However, the difference between body weight matched rats and bypass rats indicates that factors in addition to body weight or body adiposity seem to play a role [95]. Leptin is secreted from white adipose tissue, but there are differences in respect to the fat distribution concerning leptin secretion [96]. Because women have higher leptin levels than man, it has been postulated that leptin levels may correlate better with subcutaneous (female) than visceral (male) fat [97]. Hence, it is possible that the different leptin levels in bypass and body weight matched rats may have been due to altered fat distribution, but this was not assessed in our rats. A second possible explanation is that secretion activity of white adipose tissue differs between both groups.

PYY was five times elevated in bypass rats in comparison to both sham and body weight matched rats. PYY is a short-term satiation signal and may therefore play a role in mediating reduced eating and weight reduction after gastric bypass. PYY knockout mice, for example, show no direct weight reduction after gastrointestinal bypass [98]. Similar to PYY, GLP-1 secretion is usually increased after bypass surgery [99]. Surprisingly, this was not seen in our animal cohorts. However, for technical reasons and because several GLP-1 values were below the detection limit in our assay, the group sizes were too small to do reliable statistics. Hence, further studies will be necessary to define the role of GLP-1 under our experimental conditions.

For insulin, PP and GIP no differences between groups were seen. Our rats had been fasted for six hours before blood sampling; hence, the low insulin levels may have been due to the lack of food intake. The main secretion stimulus for insulin is higher blood glucose [100], but after six hours of fasting, this stimulus was probably minor. It will therefore be interesting to investigate in further experiments the potential differences in insulin secretion after food ingestion. It needs to be mentioned, however, that bypass surgery restores insulin sensitivity in diabetes patients [101] and higher insulin sensitivity may require less insulin to be secreted after bypass.
Currently, we have no explanation why GIP was not increased after bypass, as was reported before [102]. The same is true for pancreatic polypeptide [103]. Our rats may still have been relatively small and lean compared to the common bypass patient suffering from massive overweight for many years; hence, the situation may not be directly comparable.

4.6 Refeeding experiment

After bypass surgery rats decrease their food intake [104]. It has long been thought that reduced intake may be a direct consequence of an inability to ingest larger meals. However, a large number of data indicate that this is actually rather unlikely for a number of reasons. Accordingly, bypass patients often report less hunger before and increased satiation after eating [105]. Hence, we tested sham and bypass rats that had been food restricted for three days, on the day of refeeding.

Interestingly, both surgical groups had meal sizes of between two and three grams after refeeding, with no differences due to the type of surgey. At least under these conditions, bypass rats were therefore able to ingest as much food over a short period of time as sham operated rats. A second experiment with non-operated rats was then performed to test whether three restriction days were sufficient to evoke compensatory overeating in the refeeding phase; rats were divided into three groups, one control group was not restricted, one group was restricted for three days and one group for fourteen days to 50% of their ad libitum intake. To our surprise, three day restricted rats had meal sizes during refeeding that were similar to non-deprived controls, and similar to the sham and bypass rats described before. Only the fourteen day restricted rats overate markedly in the first refeeding meal by increasing meal size four folds, up to twelve grams. Hence, the comparison between sham and bypass rats may not be conclusive because the drive for compensatory overeating may not have been strong enough. Because our rats had already been in experiments for several months, we felt that it would have been inappropriate to impose such a long food restriction period, in particular in our bypass rats.

4.7 Bone density measurement

We performed density CT scans of the femurs of our bypass, sham and body weight matched rats. While bone length was the same in all groups, the bypass rats had reduced total bone density, which was due to a reduction in both cortical and trabecular density. The difference in density between bypass and sham rats can theoretically be explained by the different body weight because bone structure is altered according to the prevailing mechanical stress. However, this factor can be excluded for the differences between body weight matched and bypass rats because their weight was identical. Hence, bypass surgery seems to alter bone metabolism more specifically. One hypothesis is that reduction in bone density is simply due to malabsorptive feature after bypass surgery. Minambres at al showed in 2011 that bypass surgery evokes a severe hypocalcaemia due to vitamin D deficiency [106] and Carlin and colleagues could prevent cortical bone loss after bypass surgery in woman with weekly vitamin D administration [107]. Interestingly, Sinha at al, did a 18 month follow up study of bariatric surgery patients in 2011, which showed that bone metabolism is unregulated and that, in comparison to Carlin at al, bone density

reduction could not be prevented by vitamin D supplementation [108]. These controversies could be explained by an upregulation of vitamin D activation in order to compensate for any malabsorption [85].

Abegg and colleagues showed furthermore that initial calcium malapsorption plays a key role in bone density loss after bypass surgery. As this initial absorption decrease is normalized over time, other factors like chronic metabolic acidose may contribute in long term density loss, while the often discussed secondary hyperparathyroidism seems not to be involved [85].

4.8 Conclusion

Our rat model system of RYGB surgery showed nicely that changes in eating behavior are due to a reduction of fat preference. Most likely these changes are mediated by post prandial effects, for example the secretion of gastric peptide hormones.

Beside the effect on fat preference, RYGB surgery alters pathways of bone metabolism and thus decreases bone mass over time.

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